NEW YORK ENTOMOLOGICAL SOCIETY
LXXXV(3), pp. 153–157

TECHNIQUES FOR REARING THE ALFALFA BLOTCH LEAFMINER[1 2 3]

R. M. Hendrickson, Jr. and S. E. Barth

Abstract.—Hendrickson, R. M., Jr. and S. E. Barth, Beneficial Insects Research Laboratory, Agric. Res. Serv., USDA, Newark, Delaware, 19713.—Three techniques for rearing the alfalfa blotch leafminer, *Agromyza frontella* (Rondani), were developed. A simple method of rearing the leafminer in a cage on potted alfalfa produced small numbers of flies with a minimum of maintenance time. A second method allowed recovery of puparia (mature larvae drop from the leaflet to pupate in soil) which was useful in obtaining puparia from field-collected alfalfa stems, for some biological studies, and for rearing larval-pupal parasites. A mass rearing method, in which infested plants were laid on their sides so larvae dropped into moist vermiculite, was suited for production of large numbers of flies and efficient use of potted alfalfa. Survival of pupae, the stage during which leafminers may suffer high mortality, was 50–60% for all methods.

Received for publication 3 June 1977.

The alfalfa blotch leafminer (ABL), *Agromyza frontella* (Rondani), is a European species first found in the United States in Hampshire Co., Mass., in 1968 (Miller and Jensen, 1970). Since then it has spread through the New England states, north into the Canadian provinces of New Brunswick, Quebec, and Ontario, west to the Ohio-Pennsylvania border, and south into Maryland and eastern West Virginia. The rate of spread is such that appearance throughout the northcentral states is possible in 5–10 years.

Techniques were developed for rearing ABL so as to provide the large numbers needed both as hosts for exotic parasites and for biological studies.

Materials and Methods

All experiments were conducted at 20 ± 1.1°C and 65 ± 5% RH in continuous light. Buffalo alfalfa was used, though any variety is probably adequate. The alfalfa plants were propagated in 12.7 cm (5 in) pots in the greenhouse until they were 25–30 cm tall. Then plants were placed in standard oviposition cages (46 × 33 × 40 cm; covered with 17.7/cm (45/in) saran screen) and exposed to ca. 25 adult ♀ for 24 h, long enough to produce 1 or 2 larvae on most leaflets. Honey was provided as food. An average of 139 third-instar (mature) ABL larvae/potted plant exited from the leaflets and dropped to the soil to pupate.

Greater numbers of flies in the oviposition cage or longer oviposition

periods increased larval mortality because excessive numbers of larvae attempted to develop in a single leaflet. Also, excessive numbers of ABL adults caused large numbers of 'pinholes' (feeding punctures made by the ovipositors of female flies), which obstructed the formation of mines, and caused mortality of leafminer larvae. However, any cage of sufficient size to hold potted alfalfa and any screening fine enough to prevent escape of adult flies would probably be adequate. Very dry or very wet substrate for pupation caused high pupal mortality.

Technique 1.—After exposure in the oviposition cage, the pots of alfalfa were placed as close together as possible in standard laboratory cages. Thus most of the mature larvae dropping from leaflets (about 2 wk after oviposition) fell into pots rather than onto the cage bottom where they would die of desiccation. After all larvae had exited from the leaves, the plants were cut back so there would be some regrowth for feeding when adults emerged. In addition, fresh pots of alfalfa were added to the cages as adult flies emerged. All pots were watered daily.

The percentage survival of ABL pupae reared by this simple technique was determined by caging individual plants in a 25 cm-high plexiglas cylinder, 13 cm OD, with 0.3 cm walls. The top and the 16 ventilation holes (2.5 cm diam) drilled near the bottom of the cylinder were covered with fine organdy. The cylinder prevented larvae from leaving the pot and confined adult flies that later emerged. After all larvae had dropped into the potting mixture, the alfalfa was cut, all leaflets were removed including those which had dropped to the bottom of the pot, and the number of empty mines was counted. The adult flies that emerged were removed from the cylinder daily and counted until no further emergence took place.

Technique 2.—After alfalfa plants were removed from the oviposition cage, they were placed on benches under lights at 20°C. Eclosion and larval maturation required ca. 14 days at this temperature, so at 12 days the alfalfa was cut, and the cut stems were placed on aluminum window screen over a plastic utility tub lined with slightly moistened blotter paper (Fig. 1). (The screen prevented plant material from dropping into the tub and interfering with the collection of puparia.) The larvae emerged from the leaflets, dropped through the screen, and pupated, usually under the blotter paper. The entire unit was kept in a sealed plastic bag to maintain high humidity. However, if large water droplets condensed on the inside of the tub, some of the larvae drowned.

The puparia were lightly affixed to the bottom of the tub or to the blotter paper, but they could be dislodged without injury by washing under a gentle stream of tap water. The water with puparia in it was then poured through a fine mesh screen to isolate the puparia. To avoid desiccation, the puparia were placed in 50 mm-diam tightly-sealed petri dishes with

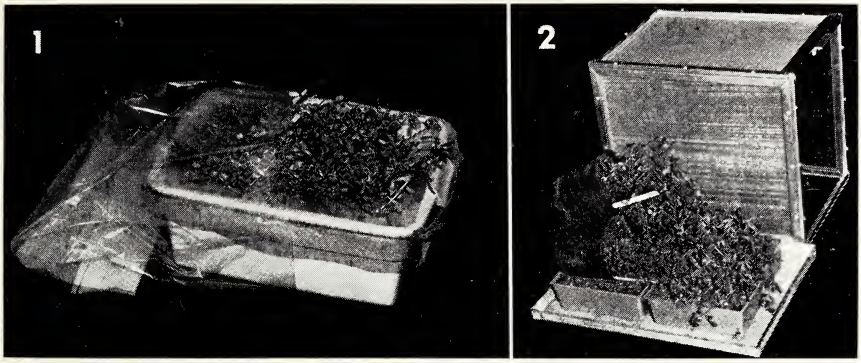

Fig. 1. Puparia recovery technique showing infested, cut alfalfa placed on window screen over plastic utility tub. Larvae drop from the plants and pupate under moistened blotter paper lining bottom of tub. High humidity is maintained by enclosing the unit in a sealed plastic bag.

Fig. 2. Mass rearing technique showing mined alfalfa plants placed on their sides so that larvae emerging from the leaflets fall into the vermiculite to pupate. Cage was removed for photograph.

moistened filter paper on the bottom. The petri dishes were examined and emerging adults removed daily.

Technique 3.—After plants were removed from oviposition cages, they were kept on benches under lights for ca. 12 days. At that time, shortly before larvae dropped from the leaflets, the plants were laid on their side on top of 4 plastic flats (13 × 20 cm) in a standard laboratory cage (Fig. 2). Each flat was filled with moistened sterilized vermiculite. Thus, the larvae dropped into the vermiculite rather than into the alfalfa pot itself. Since all the larvae developing on a given alfalfa plant were the same age within 24 h, they completed larval development as a group and emerged from the leaflets within a period of 2 or 3 days. The plants were then removed, and additional plants infested with 3rd-instar (mature) larvae were placed in the cage. Completion of the pupal stage required ca. 3 weeks, so at the end of this period the flats filled with vermiculite were placed in cages for adult emergence.

Survival of ABL pupae to adult with this mass rearing technique was determined by counting the number of empty mines from 3 plants. Adult flies were collected and counted daily.

Control of Contaminant Species.—Rearing ABL by all techniques was complicated by the presence of several arthropod pests of alfalfa. These damaged the potted plants and contaminated the ABL cultures.

Pea aphid, *Acyrthosiphon pisum* (Harris), was controlled by releasing the braconid *Aphidius ervi* Haliday[4] in the greenhouse. A culture of the parasite was maintained for this purpose. Earlier, occasional outbreaks of

pea aphid were controlled by pirimicarb (2-(dimethylamino)-5,6-dimethyl-4-pyrimidinyl dimethylcarbamate), 50% WP, applied at 0.0025% AI. This material was also used effectively against spotted alfalfa aphid, *Therioaphis maculata* (Buckton), at the same concentration. Pirimicarb caused no mortality to ABL adults or larvae.

Greenhouse whitefly, *Trialeurodes vaporariorum* (Westwood), was controlled by sprays of resmethrin ((5-benzyl-3-furyl) methyl *cis-trans*-(±)-2,2-dimethyl-3-(2-methylpropenyl) cyclopropanecarboxylate), 2% EC, applied at a rate of 0.03% AI. Alfalfa thus treated was toxic to ABL for a few days, so it was not used as host material for at least 7 days after treatment.

Organophosphate-resistant two-spotted spider mites, *Tetranychus urticae* Koch, were controlled in the greenhouse on trimmed alfalfa plants with cyhexatin (tricyclohexylhydroxystannane), 50% WP, applied at a rate of 0.0025% AI. Mites infesting fully-grown plants in the greenhouse that were ready for use or plants already in use in ABL cultures were less effectively, but adequately, controlled by Shell SD-14114 (Vendex®; hexakis (2-methyl-2-phenylpropyl) distannoxane), 50% WP, applied at a rate of 0.0025% AI. SD-14114 at this concentration caused no mortality to ABL adults or larvae or to leafminer parasites of the genera *Opius*, *Phanomeris*, *Diglyphus*, *Closterocerus*, or *Chrysocharis* which were maintained in culture on several agromyzid host species.

Results and Discussion

The most critical period in the rearing of ABL was the pupal stage, the period when mortality was uniformly high. The three techniques of rearing were ca. equally successful during this period: simple technique = 57.1% survival (n = 186), puparia recovery technique = 61.8% (n = 105), and mass rearing technique = 50.6% (n = 211).

The simple technique produced small numbers of flies with minimum maintenance time so it was convenient for rearing ABL for some biological studies or for preliminary work with parasites. The chief difficulty was that production of more than a few flies required considerable laboratory space because the pots had to be left in culture for 40 days until most adults emerged. Another difficulty was that alfalfa plants maintained under artificial lights grew more slowly and had smaller leaflets with longer internodes than alfalfa maintained in greenhouses, thus significantly reducing the leaf area of the plants. However, the simple technique could be even further simplified by eliminating the oviposition cage and keeping adult flies in cages with potted alfalfa permanently.

The puparia recovery method was particularly useful when experiments directly involved puparia, for example studying diapause, or when larval-pupal parasites were to be studied. The advantages included the

direct recovery of puparia and the rapid return of trimmed alfalfa to the greenhouse. The chief difficulty was the greater maintenance time required, more than either of the other 2 methods. This method can also be used to collect large numbers of puparia from infested field alfalfa and should be useful in collecting puparia of other agromyzid species that drop from the host plant to pupate. However, larger agromyzids such as the corn blotch leafminer, *Agromyza parvicornis* Loew, might require screen with a wider mesh than the window screen we used.

The mass rearing technique produced large numbers of flies and so was most useful when host material was needed. It required little maintenance time, made maximum use of laboratory space, and permitted rapid return of trimmed alfalfa to the greenhouse. The only difficulty was that puparia were not directly recovered, but this recovery is necessary for only a few special purposes.

Literature Cited

Miller, D. E., and G. L. Jensen. 1970. Agromyzid alfalfa leaf miners and their parasites in Massachusetts. J. Econ. Entomol. 63:1337–1338.

Footnotes

[1] Diptera: Agromyzidae.

[2] Identified by G. C. Steyskal, Systematic Entomology Laboratory, Agric. Res. Serv., USDA, c/o U.S. National Museum, Washington, DC 20560.

[3] This paper reports the results of research only. Mention of a pesticide in this paper does not constitute a recommendation for use by the USDA nor does it imply registration under FIFRA as amended. Also, mention of a proprietary product does not constitute an endorsement by the USDA.

[4] Braconid identified by P. M. Marsh, Systematic Entomology Laboratory, Agric. Res. Serv., USDA, c/o U.S. National Museum, Washington, DC 20560.